IACUC Review Meeting: **11/26/2019**

In attendance: Leslie Jarrell, Mike Narotsky, Exemption 6Exemption 6Exemption 6 and Exemption 6
Meeting was called to order by Mike Narotsky at 9:06am
Mike Narotsky has chosen Exemption 6 to replace him as DMR for an item on 11/20/2019 Agenda.
Mike will be on leave Dec 2 thru Dec 13, Exemption 6 will be acting Chair.

**20-01-002 Amendment 8:** The IACUC reviewed this document and unanimously voted to send to DMR – waive 3 Day waiting period- following FCR. DRs: Mike Narotsky and Leslie Jarrell. Discussion was as follow:

1. Section BI, Amendment Purpose, #5. "personnel" is misspelled in the first line. This is one of the only cases where we want the specific personnel named since they are being added to the ACUP; they appear to be Exemption 6 and Exemption 6.
2. #5 Purpose, paragraph 2. Installation of the OrbiTrap should be complete in November 2020 or 2019?
3. #5 Purpose, paragraph 4 and Section PRD, Procedures, #1. Only one "deep" is necessary for "plane of anesthesia".
4. Section TM, Exemption 6 and Exemption 6. State number of years with experience handling rats and mice.
5. Section PRD, #4 (Category D). Add the number of animals covered by this amendment – 16 adults?
6. PRD, #6.1 (restraint justification) and 8.1 (survival surgery justification). In these 2 paragraphs delete mention of your name and Exemption 6, and replace with "laboratory personnel as indicated in Team Member list".

*10:24am Leslie Jarrell steps out of the meeting. Quorum Maintained.*

Send all Annual Updates to DMR.
-DRs: Exemption 6 and Leslie Jarrell, if recusal needed, DRs will be Exemption 6 and Exemption 6
*10:43am Break, meeting stops.*
*10:53am Meeting begins. Quorum Maintained.*
**22-12-001 New Protocol:** The IACUC reviewed this document and unanimously voted to send to DMR following FCR. DRs: Exemption 6 and Leslie Jarrell. Discussion was as follow:
Base Info

1. Re-read the whole ACUP to ensure all elements are in the proper place, using lay language. The document sometimes uses overly technical jargon, some of which will be highlighted below, but you should also review separately to replace or define technical language whenever possible. Several sentences or paragraphs are repeated in several places. They should be stated only in the most appropriate section unless required for clarification, but typically you can write "as described in detail in section xx" where the text is secondary in importance. Points below make some suggestions. Ensure that comments you have already received are taken out of the text; for example section PD #1 (Research Project Description) has questions or comments at the end of the 2 paragraphs marked with hashtags.
2. Section PD, #1, paragraph 1. The purpose of the study should be clearer in the first paragraph. The sentence "The studies proposed can be described as adolescent and adult studies conducted with EDCs" doesn't suggest why these studies are important. In the next sentence, "matrices" is an example of a technical term which needs a lay language substitute, or at least a definition.
3. PD, #1, paragraph 3. Explain significance of vaginal opening and estrus cyclicity as important indicators of chemical estrogenic activity.
4. PD, #1, paragraph 3. Last sentence needs a semicolon: "…published toxicity data; other historical PFAS…"

5. PD, #1, paragraph 4, midway. Correct the following: "…as to whether additional chemicals will be affect the reproductive tract…"
6. PD, #1, 2nd hashtag objective. You defined LOAEL and NOAEL, but explain why they are important (for lay readers).
7. PD, #2 (Benefits of Research). Delete the last sentence ("significantly outweigh the costs to the animals").
8. PD, #3 (Research Project Approach), paragraph 1. Delete the second sentence ("Sample sizes…") – this will lead to more questions and unnecessary details about lab management.
9. PD, #3, paragraph 1, last 2 sentences. Delete "typically" from both sentences as this leads to more questions. Animals will be treated as described. If there are different treatment methods under consideration list and describe, or add by amendment later.
10. PD, #3, 1st hashtag uterotrophic assay. Delete "typically" before necropsied (could replace with approximately if necessary to indicate some variability in the timepoint). Whenever you have the word "necropsied", please precede it with the words "euthanized and". Why is there such a wide range of treatment days? Your animal accounting only indicates 1 time point per compound, so each compound should list a default number of dosing days. If studies indicate you need to test other dosing durations, you can submit an amendment for more rats and different dosing regimen.
11. PD, #3, 2nd hashtag pubertal assay. Rats will arrive at day 21 (weaning) and you will dose them on day 22, so you need to note here that you are requesting an acclimation waiver in order to perform the necessary experiments (animals typically need at least 3 days acclimation after arrival before experimental procedures are done).
12. PD , #3, Uterotrophic assay chemicals. Note that BPC and BPA are listed here but are not found in section AA (agents administered to animals) so they need to be added there unless these were added by mistake here. At the end of the list, delete "new chemicals to be tested" as all of these are new to this protocol. The "new chemicals" (EE2, E2, 8:2 FTOH, 4:2FTOH) are not spelled out as the previous ones were. 8:2 FTOH and 4:2 FTOH are listed both above and below "New chemicals to be tested" in study set 1, so again this is confusing.
13. PD, #3, Pubertal assay chemicals. As an example of repeated text, these chemicals are all the same (but in section PRD #2 it is evident that you will not test EE2 and E2 in this assay). So you can delete this list and just state that the same chemicals will be tested with the pubertal assay except for EE2 and E2 (and spell out the chemical names of those 2).
14. Section TM. For all team members under Animal Training, delete "approved" and "all CPHEA required training". So for you, your statement would read "Over 15 years of experience in animal studies". (You may want to specify rats and/or mice, or rodent studies, but animal studies is fine).
15. TM, Exemption 6 Exemption 6. Add "years": e.g. "Over 40 years of…"; "Over 10 years of…"
16. TM, Exemption 6. List # of years animal experience. She should be listed as Technical staff as well as alternate contact. Does Exemption 6 have any years of experience in animal studies? If not that's fine, just state he will be trained as you already have. List # years experience for Exemption 6 Exemption 6 Exemption 6.
17. Section PRD, #1. This section repeats some of section PD #3. We admit the two seem to be asking the same question, but you could still shorten the ACUP by writing here "The experimental design is described in Section PD #3. Further experimental details are described here" and then add only experimental design elements here that are not included in PD #3. Certainly leave out the sentence on sample size and lab management. One basic question is why do you have 6 rats per group and 5 dose levels in the adult uterotrophic assay, and 5 rats per group and 6 dose levels in the pubertal studies?
18. PRD #1, pubertal studies paragraph. Edit 3rd sentence to "Animals are euthanized and necropsied and all relevant tissues…"
19. PRD #1. Corn oil can be used for gavage studies but pharmaceutical grade sesame oil is available and needs to be used for subcutaneous injection. Add pharmaceutical grade sesame oil to the agents list.

20. PRD #1. The pubertal studies don't specify whether the rats are treated by gavage or subcutaneous injection.
21. PRD #1, "Study Set 2. Adult Pubertal Chemical PFAS Dose-Response." Here and in PRD #2, "Adult Pubertal" is used instead of just "Pubertal". It seems "adult" doesn't belong here – "female pubertal" seems appropriate. Also here under dosing period, female is misspelled.
22. PRD #12.1. For subcutaneous injections, be more specific about where the animals are injected; indicate you will use an injection map of the sites so 1 area is not injected too many times. In paragraph 1, state that the injections "shall" be administered "via sterile needle" (delete "to the"). The attached document illustrates injection sites in a rabbit, which can be used to model rat injection sites. You could do 3 injection sites over the left shoulder, the mid back on left, lumbar on left, and then the same on the right. Please indicate volume of chemical/vehicle to be injected in each site, sterility checks, and pH for each chemical. State how long the compound will be kept (does it separate out?), or how often it would be made up for injection. The reason is that some antibiotics, for example, are basic and need to be injected in a diluted form. Whichever chemicals these are may be basic or acidic and may need dilution for safe injection. Also state you will use sterile needles each time and indicate the gauge.
23. PRD #12.2. Submandibular bleeds are not acceptable for these rats – use tail vein bleeds. For tail vein bleeding, need to state the weight of animal, volume of blood collected, frequency (interval between bleeds; you state there will be up to 3 bleed days - are these 3 days in a row or something else?). The weight of the animal and the frequency of blood draws, together, will determine the volume of blood that can be taken at each blood draw. Use the following text: Animals will be held in an acrylic restrainer (or a towel wrap) for approximately 5 minutes while approximately 300 ul blood is collected from the tail vein using a butterfly needle (19G, 21G, or 23G, as appropriate for the size of the animal). To increase blood circulation, the tail will be stroked and may be warmed just prior to venipuncture by dipping in warm (not hot) tap water for up to 1 minute or held in a covered heated gelpack.
24. PRD #14.3 (and REQ animal requirements #9). Ovariectomized rats can and should be double housed when they arrive at EPA.
25. PRD #14.6. Ask Charles River for their protocol approval and describe in more detail the anesthetic regimen they use. If they do not give their detailed protocol at least ask for documentation of their IACUC approval. Charles River may provide you with more details of their surgery, anesthesia, and postoperative care procedures. Provide an overall summary here.
26. PRD #15.1., next to last sentence. Change "…animals are dealt wih humanely…" to "…animals will be treated humanely…". In Sections 15.1 and 15.2, indicate what procedures will be taken if the rats get abscesses or other skin lesions from the multiple chemical injections.
27. Section AA, Agents, add the abbreviations used for each of the chemicals in the prior sections to the Agents list here. Is estradiol not available in pharmaceutical grade?
28. Section AA. Some chemical LD50s are unknown. Consider known LD50s of similar chemical structures when planning maximum dosing levels of the unknown LD50 chemicals.
29. Section AA, PFOSA and EtPFOSA are listed in section AA, but are not listed elsewhere in the ACUP. Delete or describe their use.
30. Section EU, #1. State that a backup guillotine will be available for use.

*10:59pm* **Exemption 6** *returns to the meeting. Quorum Maintained.*
*11:00am Meeting stops, had to change rooms.*
*11:05am Meeting begins. Quorum Maintained.*

12:10pm Meeting Adjourned

IACUC Review Meeting: **12/18/2019**

In attendance: Leslie Jarrell, Mike Narotsky, Exemption 6, Exemption 6, Exemption 6, Exemption 6, and Exemption 6

Meeting was called to order by Mike Narotsky at 1:06 PM.
Agenda:
Items: 2,3,5,1, and 4

**Item 2 & 3**:
**20-10-001 Amendment 5 + 6:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Leslie Jarrell. Discussion was as follow:
The committee felt that the best approach for dealing with these amendments is to consolidate them into one amendment; i.e**., let's place all of the material from Amendment 6 into Amendment 5**. (Subsequently, Amendment 6 can be withdrawn.)
Throughout, check spelling (e.g., sufficient, preliminary, compounds, methoxyfenozide).
Amendment Purpose
Note: After the meeting, I looked over your previous amendments and discovered that Amendment 1 had already added mepanipyrim (and triclocarban by the way), with a maximum dose of 500 mg/kg. Given that amendment 6 is attempting to add mepanipyrim as a new compound with a maximum dose of 1000 mg/kg, I am assuming that rather than add this chemical, you wish to increase the maximum dose from 500 mg/kg to 1000 mg/kg. (If I assume incorrectly, please adjust accordingly.)

Update the opening statement to include everything from both original amendments. Be succinct and specific. Something like…
The purpose of this amendment is:

1) Increase the maximum dose of four compounds (mepanipyrim, methoxyfenozide, etoxazole, and cyprodinil) from 500 mg/kg to 1000 mg/kg
2) Add one compound: perfluorohexanesulfonic acid (PFHxS)

For the four compounds with the increased maximum dose:

- Provide justification for increasing the dose.
    - For methoxyfenozide, the current maximum dosage (500 mg/kg) has been tested and did not cause a response.
    - For etoxazole and cyprodinil, based on results from previous pilot studies…
        - Please rephrase "Gastro Plus Modeling data" with lay language.
    - For mepanipyrim?
- provide text regarding expected effects (as in previous amendment): "…these increased doses are not expected to produce any symptoms of toxicity that would require humane intervention as based on previous dose response data and findings reported in literature."

For the new chemical:

- Give background on the chemical… How is it used commercially? Is it in the environment? How are humans exposed?
- provide dosing information a la the parent protocol:
    - As described in the parent protocol, test chemicals will be prepared in methyl cellulose, corn oil, or nano-pure water depending upon solubility. All doses will be administered by oral gavage (one dose per day between 7 - 9 AM), and dosing volume will be based on the daily body weight of each rat at 2 ml/kg. For chemicals with poor solubility, the dosing volume may be increased up to 5 ml/kg body weight. Doses used are not expected to produce any symptoms of toxicity that would require humane intervention as based on previous dose response data and findings reported in literature.
- Does it need an HSRP? If so, provide info in the chemical table.

Update the chemical table for all five chemicals.

6. Assurance that Study is Not Unnecessary Duplication
Do a literature search to confirm that there is no unnecessary duplication for the new chemical.
If you keep the existing text, please spell out, define, or remove "HTS RAIU".

**Item 5**:
**20-05-001 Amendment 3:** The IACUC reviewed this document and unanimously voted to send to DMR – waive the 3 day wait-following FCR. DRs: Mike Narotsky and Exemption 6. Discussion was as follow:
Amendment Purpose

- After "ACUP 22-03-001" please insert the title of that protocol: Fetal and postnatal assessment of in utero and lactational exposures to endocrine disrupting chemicals in rats.
- Indicate that at necropsy, a backup guillotine will be available.

Team Members

- For Exemption 6 Exemption 6, please include "euthanasia" as a responsibility.

Procedures

- Indicate 40 adults for Category C

**Item 1**:
**22-12-002 New Protocol:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Leslie Jarrell. Discussion was as follow:
-Section PD- Project Description

- Neonatal is misspelled (second sentence).
- Define PND at first use (third sentence).
- Second paragraph. Please add brief descriptions of open field and elevated zero maze tests, and provide more details in the procedures section.
- Third paragraph:
    - Suggest inserting "and their litters" after "pregnant dams" so it reads "using 6 pregnant dams and their litters…" and later "we will use 16 pregnant dams and their litters…"
    - In the next sentence, change "This data…" to "These data…"
    - Last sentence: Change time-pregnant to timed-pregnant.
- Timeline
    - 1. Define GD: Dams arrive on gestation day (GD) 9. (I MUCH prefer "gestation day" vs. gestational day, but both are used.)
    - 5 & 6. Insert "to" after "believed".
    - 5 & 6. Suggest deleting "collected and" prior to recorded. Unclear how vocalizations are "collected'. Just say they are recorded.
- Last paragraph. Suggest changing "sacrificed" to "euthanized".

Section TM – Team Members For each team member's Animal Training, delete "All CPHEA training completed." (The NHEERL Training = Yes feature takes care of this.)

- For Exemption 6 role, include "Technical Staff" in addition to Alternate Contact.
- The entry for Exemption 6 Exemption 6 should be fixed. She should be in the database (note the correct spelling of her first name). Her organization will probably be the same as for Exemption 6 Exemption 6.
- For Exemption 6, please correct the spelling of experience, experimental, and approximately.

Section PRD – In Vivo Procedures

- Third paragraph:
    - Hyphenate "single-sex groups"
    - Next sentence, instert "be" …the dam will be placed into…
    - Define (or spell out) IR
    - Microphone is missing the "e" …recorded for three minutes from a microphonE suspended above…

- Section 12.4. Please change "Pups/former pups…" to "Offspring"
- Section 15.2.
    - Include dystocia and vaginal bleeding as signs of ill health.
    - Please change the ending of the last sentence so it reads "…the AV will be consulted".

Section REQ – Animal Requirements

- Section 9.
    - First sentence doesn't make sense. How about changing to "…will be housed with their dam and litter as a group until weaning (PND 22).
    - "separated" is misspelled.
    - "will" is duplicated.
    - Indicate that pregnant dams and litters will be provided nesting material (e.g., Enviro-dri or Bed-R-Nest).

Section AA – Agents Administered

- For CO2, change volume to "To effect"

Section EU – Euthanasia

- Section 1.
    - Give the approximate age that the offspring will be euthanized.
    - For CO2, change volume to "To effect"
    - For Decapitation, Death Confirmation Description, please change this to "Vital organ section" or "Exsanguination"
- Section 2
    - Please change "former pups" to "offspring" or "adult offspring"

*2:20pm Meeting Break. Meeting stopped*
*2:30pm Meeting reconvenes. Quorum Maintained.*

**Item 4**:

**22-12-001 New Protocol:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. Correspondence: Exemption 6, DRs: Mike Narotsky and Leslie Jarrell. Discussion was as follow:

1. Re-read the whole ACUP to ensure all elements are in the proper place, using lay language. The document sometimes uses overly technical jargon, some of which will be highlighted below, but you should also review separately to replace or define technical language whenever possible. Several sentences or paragraphs are repeated in several places. They should be stated only in the most appropriate section unless required for clarification, but typically you can write "as described in detail in section xx" where the text is secondary in importance. Points below make some suggestions. Ensure that comments you have already received are taken out of the text; for example section PD #1 (Research Project Description) has questions or comments at the end of the 2 paragraphs marked with hashtags.
2. Section PD, #1, paragraph 1. The purpose of the study should be clearer in the first paragraph. The sentence "The studies proposed can be described as adolescent and adult studies conducted with EDCs" doesn't suggest why these studies are important. In the next sentence, "matrices" is an example of a technical term which needs a lay language substitute, or at least a definition.
3. PD, #1, paragraph 3. Explain significance of vaginal opening and estrus cyclicity as important indicators of chemical estrogenic activity.
4. PD, #1, paragraph 3. Last sentence needs a semicolon: "…published toxicity data; other historical PFAS…"
5. PD, #1, paragraph 4, midway. Correct the following: "…as to whether additional chemicals will be affect the reproductive tract…"
6. PD, #1, 2nd hashtag objective. You defined LOAEL and NOAEL, but explain why they are important (for lay readers).
7. PD, #2 (Benefits of Research). Delete the last sentence ("significantly outweigh the costs to the animals").
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14. Section TM. For all team members under Animal Training, delete "approved" and "all CPHEA required training". So for you, your statement would read "Over 15 years of experience in animal studies". (You may want to specify rats and/or mice, or rodent studies, but animal studies is fine).
15. TM, Exemption 6. Add "years": e.g. "Over 40 years of…"; "Over 10 years of…"
16. TM, Exemption 6. List # of years animal experience. She should be listed as Technical staff as well as alternate contact. Does Exemption 6 have any years of experience in animal studies? If not that's fine, just state he will be trained as you already have. List # years experience for Exemption 6.
17. Section PRD, #1. This section repeats some of section PD #3. We admit the two seem to be asking the same question, but you could still shorten the ACUP by writing here "The experimental design is described in Section PD #3. Further experimental details are described here" and then add only experimental design elements here that are not included in PD #3. Certainly leave out the sentence on sample size and lab management. One basic question is why do you have 6 rats per group and 5 dose levels in the adult uterotrophic assay, and 5 rats per group and 6 dose levels in the pubertal studies?
18. PRD #1, pubertal studies paragraph. Edit 3rd sentence to "Animals are euthanized and necropsied and all relevant tissues…"
19. PRD #1. Corn oil can be used for gavage studies but pharmaceutical grade sesame oil is available and needs to be used for subcutaneous injection. Add pharmaceutical grade sesame oil to the agents list.
20. PRD #1. The pubertal studies don't specify whether the rats are treated by gavage or subcutaneous injection.
21. PRD #1, "Study Set 2. Adult Pubertal Chemical PFAS Dose-Response." Here and in PRD #2, "Adult Pubertal" is used instead of just "Pubertal". It seems "adult" doesn't belong here – "female pubertal" seems appropriate. Also here under dosing period, female is misspelled.
22. PRD #12.1. For subcutaneous injections, be more specific about where the animals are injected; indicate you will use an injection map of the sites so 1 area is not injected too many times. In paragraph 1, state that the injections "shall" be administered "via sterile needle" (delete "to the"). The attached document illustrates injection sites in a rabbit, which can be used to model rat injection sites. You could do 3 injection sites over the left shoulder, the mid back on left, lumbar on left, and then the same on the right. Please indicate volume of chemical/vehicle to be injected in each site, sterility checks, and pH for each chemical. State how long the compound will be kept (does it separate out?), or how often it would be made up for injection. The reason is that some antibiotics, for example, are basic and need to be injected in a diluted form. Whichever chemicals these are may be basic or acidic and may need dilution for safe injection. Also state you will use sterile needles each time and indicate the gauge.
23. PRD #12.2. Submandibular bleeds are not acceptable for these rats – use tail vein bleeds. For tail vein bleeding, need to state the weight of animal, volume of blood collected, frequency (interval between bleeds; you state there will be up to 3 bleed days - are these 3 days in a row or something else?). The weight of the animal and the

frequency of blood draws, together, will determine the volume of blood that can be taken at each blood draw. Use the following text: Animals will be held in an acrylic restrainer (or a towel wrap) for approximately 5 minutes while approximately 300 ul blood is collected from the tail vein using a butterfly needle (19G, 21G, or 23G, as appropriate for the size of the animal). To increase blood circulation, the tail will be stroked and may be warmed just prior to venipuncture by dipping in warm (not hot) tap water for up to 1 minute or held in a covered heated gelpack.

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25. PRD #14.6. Ask Charles River for their protocol approval and describe in more detail the anesthetic regimen they use. If they do not give their detailed protocol at least ask for documentation of their IACUC approval. Charles River may provide you with more details of their surgery, anesthesia, and postoperative care procedures. Provide an overall summary here.
26. PRD #15.1., next to last sentence. Change "…animals are dealt wih humanely…" to "…animals will be treated humanely…". In Sections 15.1 and 15.2, indicate what procedures will be taken if the rats get abscesses or other skin lesions from the multiple chemical injections.
27. Section AA, Agents, add the abbreviations used for each of the chemicals in the prior sections to the Agents list here. Is estradiol not available in pharmaceutical grade?
28. Section AA. Some chemical LD50s are unknown. Consider known LD50s of similar chemical structures when planning maximum dosing levels of the unknown LD50 chemicals.
29. Section AA, PFOSA and EtPFOSA are listed in section AA, but are not listed elsewhere in the ACUP. Delete or describe their use.
30. Section EU, #1. State that a backup guillotine will be available for use.

*2:51pm* Exemption 6 *leaves meeting. Quorum maintained.*
*2:55pm* Exemption 6 *returns to meeting. Quorum Maintained*

New Business:

- Annual updates were submitted and sent to DMR.
- Rabbits will need more clipping of fur. Will need to be anesthetized to clip.
- Born Onsite information needs to be counted. Upper Management has requested pups need to be counted.

*4:28pm Leslie Jarrell leaves meeting. Quorum maintained.*

4:31pm Meeting Adjourned

IACUC Review Meeting: **01/15/2020**

In attendance: Leslie Jarrell, Mike Narotsky, Exemption 6Exemption 6Exemption 6 Exemption 6, and Exemption 6

Meeting was called to order by Mike Narotsky at 2:10 PM.

New Business:

1. AAALAC Site Visit is scheduled for February 28, 2020
2. Mock Site Visit is scheduled for February 05, 2020
3. Rabbits are doing well. Wyatt has been clipped and cleaned. Had to be anesthetized to do the procedure.
4. Rat Oral Gavage Class
    a. 1 complication from the class
    b. More training is on the schedule
5. Exemption 6 Exemption 6 Exemption 6 Rats are still scuffling
    a. The Rats get along during the day as they sleep.
    b. The get into scuffles at night
    c. Trying to mitigate, will try splitting the pens and place in different rooms
    d. Reduce pheromones and stimulation
6. VAV renovation will be shutting down cage wash.
    a. Possible Portable AC units for chilling the fish
    b. Disposable PET Plastic cages for studies in case of emergency.

Action Item: Send out Policies and Procedures to the Health IACUC. Check to see if we need to meet to continue to use them.

Agenda:
Items: 1 and 2

**Item 1**:
**21-08-003 Amendment 5:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Exemption 6. Discussion was as follow:

**Project description:**

- Please mention the reason for adding these 36 chemicals for testing in terms of known adverse effects.
- Are the concentrations being tested (highest conc is 100 uM) are environmentally and biologically relevant?
- Please provide headers or explanation for three columns in the table.
- Why are both sodium and potassium salts of Perfluorooctanoate being tested?
- Can the salts themselves have an impact?

**Assurance that study is not …….:**

- It would be nice to have dates the search was done.
- Was MSDS a source of tox data search for research purpose? Probably, should remove it.

**Animal Requirements:**

- Please move numbers in 4 (2592) to 3 and then list the Breeding protocol number. No transfer form is expected.

**Agents administered to Animals:**

- The justification given for pharmaceutical grade is ok, but good justification is that they are not available in pharmaceutical grade. Please add this.
- MS222 is available in pharmaceutical grade. Please add this.
- Please change justification for Bleach about pharmaceutical grade. Not likely prepared by another lab. Please mention that it is not available in pharmaceutical grade. Mention that Rapid chilling complies with AVMA guidelines.
- Change LAPR to ACUP.
- The section "Other" does not belong in Euthanasia. Please remove.

**Item 2**:
**23-01-001 New Protocol:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Exemption 6. Discussion was as follow:

Editorial changes throughout

- Please replace "sacrifice", "sacrificed", etc. with "euthanize", "euthanasia", etc. (or "kill").
- Change "five days" to "5 days". (Use numerals for units of measure.)
- Change "eight doses" to "eight dose levels" or "eight dosages". (This is for clarity; in some contexts, "eight doses" can be inferred to mean 8 days of gavage.)

Section PD – Project Description

- Section 1 needs lay language. Phrases like "gene expression" need to be explained in non-technical language.
- MUCH more information is needed here. It is unclear what the real purpose of this protocol is…. Is it to learn about these two chemicals, or is it to refine an assay? Is this testing method well established, or is this a proof-of-concept?
- There are thousands of data-poor chemicals; why select these two chemicals? Explain the rationale for selecting these two chemicals. What are they used for, or how are humans exposed, or how are they found in the environment???
- It part of the purpose of this work is to demonstrate or refine the testing method, this work would be strengthened with a positive control. I.e. test a third chemical that you know would cause the long term effect (cancer?), and see if it causes the predicted changes in gene expression after 5 days of dosing. (Has this already been done?)
- Please better justify the 10% change in gene expression as an estimate at which adverse effects occur. Is 10% change enough to be predictive? (Perhaps a provide a reference?) Is this a "general" phenomenon, or a gene-specific phenomenon?
- Section 3. Euthanasia methodological details aren't necessary here; please delete.
    - That said, if little is known about these chemicals, it would be a an opportunity to learn as much as possible from these animals. I.e., PLEASE preserve as many tissues as practical for subsequent histopathological examination (e.g., liver, kidney, heart, lung, trachea, gonads, reproductive tissues, mammary). Also, take blood for measures of liver and kidney damage (e.g., SGOT, SGTP, creatinine).

*3:37pm Call PI on the phone to help answer questions.*

Section TM – Team Members

- Remove folks that won't be handling live animals (i.e., Exemption 6). If they have had barrier training, they can still have badge access to the vivarium and assist with necropsies. (Note: Exemption 6 has not had AALAS or hands-on animal training; therefore, until she completes the necessary training, she is not permitted to euthanize animals.)

- Please indicate the years' experience, under Animal Training, for [redacted] (>20 years), [redacted], and [redacted].
- [redacted] is listed as [redacted], but in one instance referred to as [redacted]. To avoid confusion regarding the name change, it might be simpler to just say "[redacted]".

Section PRD – In Vivo Procedures

Section 1. Experimental Design

- Change "quarantine" to "acclimation". (No need for quarantine; the animals will not be quarantined.)
- Highly recommend buying animals much younger (3 weeks old). This provides several advantages (saves money; avoids inter-male aggression; handling will make gavage easier).
- Pine bedding (even heat-treated pine) releases terpenes which can affect liver function and will likely affect gene expression. Suggest different bedding.
- Remove details regarding dosing and euthanasia methodology. (This section is for the big picture. Details come later.)
- Remove necropsy details. This level of postmortem methodology is not necessary in an ACUP.
- If there is no IN VIVO dose-response information for these chemicals, conduct a dose-range-finding study before launching into the study as currently proposed. There are several acceptable approaches to conducting a dose-range-finding (DRF) study…
    - One possible DRF approach: Dose two animals of one sex at a selected dosage (e.g., 1000 mg/kg) with two concurrent controls. If there are clinical effects, select a lower dosage (e.g., 500), and try again. At the dose level where the chemical-treated animals survive 5 days without issue, the animals can be necropsied and used for full data collection. The other sex can then be tested to ensure no sex differences in sensitivity.
        - This proposed approach would require up to 36 animals per chemical (e.g., 32 males, 4 females). (Each dosage would have its own concurrent controls.)
        - Also present DRF study design in Section PRD-1. Request additional animals for DRF studies in Section PRD-2.

Section 2. Number of Animals Justification

- Justify the sample size (n=4) per group. Is the data variability low enough to provide reliable BMDL values?
- Request additional animals if needed for dose-range-finding studies. Show justification.

Section 12.7. Describe how animals will be…monitored

- Animals need to be monitored EVERY day after dosing, including weekends.
- Indicate that animals will be monitored after dosing morning and afternoon every day after dosing (including weekends), and that a data log will be maintained. The weekend afternoon observation session can be skipped only when it has been established that the chemical has minimal clinical effects at the doses being tested.

Section 15.1. Resultant effects

- Change the last two sentences:
    - Therefore, the attending veterinarian (AV) will be contacted if animals are experiencing lethargy, ataxia, salivation, tremors, or other clinical signs of ill health or distress. If necessary, after AV consultation, the animals will be humanely euthanized.

Section 15.2. …criteria for…removal…

- Add two sentences…
    - If there is a pattern of clinical effects, removal of the entire treatment group from the study may be warranted. If necessary, after AV consultation, animals will be euthanized.

Section REQ – Animal Requirements

- Adjust numbers as necessary to accommodate dose-range-finding studies.

- Section 9. Housing, Husbandry, Enrichment
    - Suggest different bedding rather than heat-treated pine.
    - Delete sentence on temperature and humidity.
    - Is 20% weight variation refer to the beginning of the study? What if you have outliers?
- Section 10. Special Assistance
    - You'll need to submit a Technical Service Request for oral gavage assistance.
        - Note that performance of a TSR is based on availability.
        - There will need to be a preparatory meeting with Animal Care Staff (ACS) prior to performing the gavage to discuss timing, safety, handling toxic waste, etc.
        - The lab staff is expected to do the daily monitoring. ACS will help, but it is not their specialty.

Section AA – Agents Administered

- For both test articles, delete "levels" after "pharmaceutical grade"
- For CO2, change dose to "To effect". Change volume to "Approximately 50% displacement of 8 liters/minute". (The new AVMA Euthanasia Guidelines just came out, increasing the flow rate.)

Section EU – Euthanasia

- For CO2, change dose to "To effect". Change volume to "Approximately 50% displacement of 8 liters/minute". (The new AVMA Euthanasia Guidelines just came out, increasing the flow rate.)

*4:06pm Leslie Jarrell leaves meeting. Quorum maintained.*

4:35pm Meeting Adjourned

IACUC Review Meeting: **02/12/2020**

In attendance: Leslie Jarrell, Mike Narotsky, Exemption 6 (via phone), Exemption 6 Exemption 6, Exemption 6, and Exemption 6

Meeting was called to order by Mike Narotsky at 1:08 PM.

New Business:

1. AAALAC Site Visit is scheduled for February 28, 2020
2. Exemption Exemption Exemption Exemption Rats-2 high dose rats adverse event from this weekend
3. Exemption 6 Exemption 6 Exemption 6 Rats-1 adverse event. Will change cages more frequently The adverse event rat was taken off study.
4. Exemption 6 Exemption 6 Exemption 6 Rats Study is finishing up soon.

Agenda:
Items: 2 then 1

**Item 2**:
**23-02-001 New Protocol:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Exemption 6. Discussion was as follow:

1. The following edited ACUP title might be a little more straightforward – please consider: "Discovery of Proteomics-Based Adverse Outcome Pathways for Neurotoxicity Using Cultured Rat Neurons".
2. Section PD, #1 Research Project Description. This is an introduction to the research with nicely written lay language, but the goal here is to describe the study objectives, which are not really evident in this section. The first 2 paragraphs of #2 (Benefits Proposed Research) seems to more clearly describe your objectives, so these could be moved up to #1, but be sure these represent the best description of study objectives.
3. PD #1, 3rd paragraph. Correct error in number "100,0000".
4. PD #2. Under Benefits of Proposed Research, it would be helpful to add a sentence about this research potentially leading to reduced need for animal testing and quicker toxicity testing.
5. PD #3 (Research Project Approach). Much of this text seems like a rationale for the study rather than a description of the experimental design, which is the question here. You may want to move some of this text to the introduction in PD, #1, but be sure to add some text here on the experimental design used to meet the objectives. Last word of this paragraph: replace "ACUP" with "protocol".
6. PD #4. The sentence beginning "In fact, this research is being planned…" does not address the question about unnecessary duplication, so it should be deleted. Add here keywords and years covered of the search.
7. Section TM. Select the team Member who handles euthanasia of rat pups and indicate this task under Responsibilities. Send IACUC representative the training certificate from whoever trained the person on decapitation.
8. Section PRD #2 Number of animals justification. About 4 lines from end of this section, edit "process in which kills" to "process which kills". Do spell-check search for "liter" which is used here twice instead of "litter" and is found also in several other sections. Describe the litter effect more fully – littermates are more similar to each other and not independent.
9. Section PRD #12.1 Edit "They are decapitated…" to "The pups are decapitated…" Next sentence delete "in good shape", and mention a spare pair of sharp scissors will be available.

10. Section REQ #9. State that pups will be kept warm in transit to the lab – how will this be ensured on trip to lab? Why not kill pups in A 2nd floor? Correct spelling of "liter" (twice) and "varibility ".
11. Section EU #1. Correct spelling of "decapitaed". In physical euthanasia confirmation description, correct spelling of "doen".
12. It is worth enquiring with Exemption 6 if he will be willing to work with you on using pups from the same litter, as he only uses a couple and you only use 4 pups, in order to reduce the numbers of research animals used.

**Item 1**:
**Annual Update:** The IACUC reviewed this document and unanimously voted to postpone approving the current annual update and send comments to the programmers of ACUP as the IACUC could not view all the information of the Annual Update. The questions were not formatted onto the downloaded PDF. Exemption 6 will contact the ACUP Programmers to find out why the template for the Annual Update does not print.

2:56pm Meeting Adjourned

IACUC Review Meeting: **02/26/2020**

In attendance: Mike Narotsky- Chair, Leslie Jarrell, Exemption 6Exemption 6 Exemption 6 Exemption 6 Exemption 6, and Exemption 6

Meeting was called to order by Mike Narotsky at 1:10 PM.

New Business:

1. AAALAC Site Visit is scheduled for February 28, 2020

Agenda:
Items: 1 and 2 (3)

**Item 1**:
**21-04-002 Amendment 3:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Exemption 6. Discussion was as follow:

-Waiting on the correspondence to check notes (Narotsky or Exemption 6 Exemption 6 Exemption 6)

*1:37pm* Exemption 6 *recuses and leave meeting. Quorum Maintained.*
**Item 2**:
**20-08-003 Amendment 11:** The IACUC reviewed this document and unanimously voted to approve with administrative changes. Exemption 6 will make the administrative changes.

Base Information #5

- 2nd Paragraph, change have to has
- Add HSRP in front of #902

Section REQ #3

- Replace 0 with 6577 and add protocol number

Section REQ #4

- Replace 6577 with 0

**Item 3**:
**Annual Update:** Will not review as the formatting of the document is still not right. Will push to the next Health IACUC Agenda.

1:46pm Meeting Adjourned

IACUC Review Meeting: **03/11/2020**

In attendance: Mike Narotsky- Chair, Leslie Jarrell, Exemption 6Exemption 6, Exemption 6, and Exemption 6

Meeting was called to order by Mike Narotsky at 2:30 PM.

Agenda:
Items: 1
**Item 1**:
**21-04-002 Amendment 4:** The IACUC reviewed this document and unanimously voted to send to DMR - following FCR. DRs: Mike Narotsky and Exemption 6. Discussion was as follow:
Amendment Purpose

- Add "in order to evaluate the accumulation of perfluorinated chemicals in the liver"
- Change The to This
- Be more specific about filtration. What about microbial concerns? Will filtration also remove microbes?
- Please clarify… All animals, including control, receive the MC mixture? Or, just the envirowater animals receive the MC mixture?
- Please lay out the experimental design.
- Clarify the rationale for the MC mixture.
- Wouldn't the envirowater + MC exposure group be more meaningful if there was an envirowater group WITHOUT the MC mixture to compare against?
- The parent protocol has ip administration, and gavage administration. Just double checking… which route will be used here?
- Replace toxins with toxicants

Section REQ – Animal Requirements

- Section 1 Animal Justification
- Animal number to: (56 males and 56 females)
- Treated vivarium water?
- The parent protocol has preliminary studies (6 animals per group) and definitive studies (10 animals per group). Is that what's happening here? Assuming no, remove "definitive"
- Here and/or in Section BI-5, very clearly lay out the experimental design. (E.g., Do controls get the MC mixture?)
- Add how you calculated the animal numbers: 12 control (6 male, 6 female) + 20 treated (10 male, 10 female) = 32 mice/study 3 studies x 32 mice/study = 96 mice + 16 mice (8 male, 8 female) for unanticipated events = 112 mice total

*2:56pm Leslie Jarrell left the meeting. Quorum Maintained.*

3:15pm Meeting Adjourned